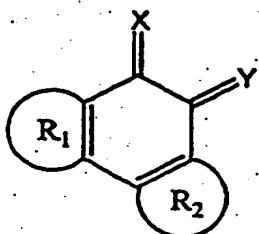


## REMARKS

Claims 1-5 and 7-11 remain in the application. Claims 1, 3, 4, 7, 9, and 10 have been amended. A version with markings to show changes made follows page 13. Claims 6 and 12-15 have been cancelled. Claims 16-29 have been added. Reconsideration of this application, as amended, is respectfully requested.

Claim 1 has been rewritten to recite that the mediator compound has the following formula



where  $X$  and  $Y$  can independently be oxygen, sulphur,  $CR^3R^4$ ,  $NR^3$ , or  $NR^3R^4$  or the functional group  $CZ^1Z^2$ , where  $Z^1$  and  $Z^2$  are electron withdrawing groups;  $R_1$  is a six-membered unsubstituted or substituted heteroaromatic ring having nitrogen in the 1-position and  $R_2$  is a six-membered unsubstituted or substituted heteroaromatic ring having nitrogen in the 10-position; and  $R^3$  and  $R^4$  can independently be a hydrogen atom, a hydroxyl group or a substituted or unsubstituted alkyl, aryl, heteroaryl, amino, alkoxy, or aryloxy group

Support for this amendment can be found at page 3, lines 2-4 and at page 5, lines 1-3 of the specification and in claim 2, as originally filed. Claim 1 has been further amended to delete the recitation "such that said electrode gives a monotonic response to concentrations of said analyte between 1 and 8 mM when measurement is made in a kinetic mode in which simultaneous oxidation and reduction of the mediator occurs during the measurement." In view of the changes made to claim 1, this feature is not required.

Claims 3 and 4 have been amended to depend from claim 1. Claims 7, 9, and 10 have been amended to depend from claim 5.

Claim 16 has been added to recite that  $R_1$  or  $R_2$  or both  $R_1$  and  $R_2$  can be

substituted with at least one alkyl group. Claim 17 has been added to recite that R<sub>1</sub> or R<sub>2</sub> or both R<sub>1</sub> and R<sub>2</sub> are unsubstituted. Support for these claims can be found at page 5, lines 2-4 of the specification.

Claims 18, 20, 21, 24, 25, 27, 28, and 29 have been added to provide claims analogous to claims 1, 3, 4, 5, 7, 9, 10, and 11, respectively, with the exception that the substituents X and Y are located para to each other rather than ortho to each other. Support for these claims can be found at page 2, lines 14-17 of the specification and in claims 1 and 5, as originally filed.

Claims 19 and 26 have been added to provide claims analogous to claims 2 and 8, respectively, with the exception that the substituents X and Y are located para to each other rather than ortho to each other. Claims 22 and 23 have been added to provide claims analogous to claims 16 and 17, respectively, with the exception that the substituents X and Y are located para to each other rather than ortho to each other.

Claims 6 and 7 were rejected under 35 U. S. C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 6 has been cancelled. Claim 7 has been amended to depend from claim 5. Accordingly, this rejection can be withdrawn.

Claims 1-3 and 12 stand rejected under 35 U. S. C. § 103(a) as being unpatentable over Geng et al., "Amperometric biosensors based on dehydrogenase/NAD and heterocyclic quinones", *Biosensors & Bioelectronics*, Vol. 11, No. 12, pp. 1267-1275, 1996, in view of MacFarlane et al. (USP 5,212,622) and Carter et al. (USP 5,628,890). This rejection is respectfully traversed for the following reasons.

Geng et al., "Amperometric biosensors based on dehydrogenase/NAD and heterocyclic quinones", *Biosensors & Bioelectronics*, Vol. 11, No. 12, pp. 1267-1275, 1996, (hereinafter "Geng et al."), discloses the electrocatalytic oxidation of NADH with heterocyclic quinones dissolved in a water solution. The heterocyclic quinone used was 1,7-phenanthroline-5,6-dione.

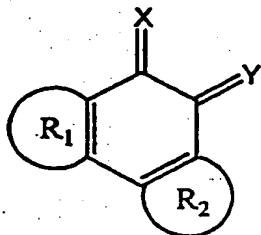
MacFarlane et al., U.S. Patent No. 5,212,622 (hereinafter "MacFarlane et al."), discloses a high surface area electrode comprising a composite of an

electronically conductive particulate filler dispensed in a binder material, wherein the particles are substantially in intimate contact, and the binder material is able to support conduction of ions to and from a substantial proportion of the surface of the filler particles. The binder material may comprise a polymeric material having ionized groups and corresponding counterions.

Carter et al., U. S. Patent No. 5,628,890 (hereinafter "Carter et al."), discloses an electrode strip for use in an electrochemical sensor for measuring a compound in a sample, including an electrode support, a reference or counter electrode disposed on the support, a working electrode spaced from the reference or counter electrode on the support, a covering layer defining an enclosed space over the reference and working electrodes and having an aperture for receiving a sample into the enclosed space, and a plurality of mesh layers interposed in the enclosed space between the covering layer and the support, the covering layer having a sample application aperture spaced from said electrodes and said reference electrode spaced from said working electrode at a position remote from and on the opposite side of said working electrode from said aperture. The working electrode includes an enzyme capable of catalyzing a reaction involving a substrate for the enzyme or a substrate catalytically reactive with an enzyme and a mediator capable of transferring electrons transferred between the enzyme-catalyzed reaction and the working electrode to create a current representative of the activity of the enzyme and representative of the compound.

The electrode strip of the present invention requires that the mediator must be either:

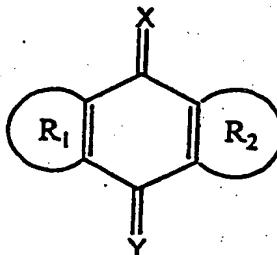
(a)



where X and Y can independently be oxygen, sulphur, CR<sup>3</sup>R<sup>4</sup>, NR<sup>3</sup>, or NR<sup>3</sup>R<sup>4</sup> or the functional group CZ<sup>1</sup>Z<sup>2</sup>, where Z<sup>1</sup> and Z<sup>2</sup> are electron withdrawing groups; R<sub>1</sub> a six-membered unsubstituted or substituted heteroaromatic ring

having nitrogen in the 1-position and R<sub>2</sub> is a six-membered unsubstituted or substituted heteroaromatic ring having nitrogen in the 10-position; and R<sup>3</sup> and R<sup>4</sup> can independently be a hydrogen atom, a hydroxyl group or a substituted or unsubstituted alkyl, aryl, heteroaryl, amino, alkoxy, or aryloxy group, or

(b)



where X and Y can independently be oxygen, sulphur, CR<sup>3</sup>R<sup>4</sup>, NR<sup>3</sup>, or NR<sup>3</sup>R<sup>4</sup> or the functional group CZ<sup>1</sup>Z<sup>2</sup>, where Z<sup>1</sup> and Z<sup>2</sup> are electron withdrawing groups; R<sub>1</sub> is a six-membered unsubstituted or substituted heteroaromatic ring having nitrogen in the 1-position and R<sub>2</sub> is a six-membered unsubstituted or substituted heteroaromatic ring having nitrogen in the 8-position; and R<sup>3</sup> and R<sup>4</sup> can independently be a hydrogen atom, a hydroxyl group or a substituted or unsubstituted alkyl, aryl, heteroaryl, amino, alkoxy, or aryloxy group

Geng et al. discloses that there is no catalytic current for 1, 10-phenanthroline quinone. See for example, the following passages in Geng et al., in which I represents 1,7-phenanthroline-5,6-dione and II represents 1,10-phenanthroline-5,6-dione.

Cyclic voltammetry of II was similar to the cyclic voltammetry of I, but no catalytic activity of NADH oxidation was observed in the water buffer solution (Fig. 2(A)). In a water ethanol (1:1) mixture the effect of NADH on catalytic current is also negligible (Fig. 2(B)). This fact could be explained by the formation on the electrode surface of a stable, poorly oxidizable reduce form of II. A special experiment with addition of NADH to the solution of quinone II, when both reagents were taken at higher concentrations, demonstrated the precipitation of the reaction product. This fact explains the decreasing current

amplitude shown by the dotted line in Fig. 2(A). This experiment indicates, that if the reduced form of II exists on the surface, it is not active electrochemically. (see last paragraph, column 2, page 1269 of Geng et al.)

The radical form of II is more stable to oxidation on the electrode. In addition, in water solution the intermediate II is poorly soluble and partly precipitate. Therefore, after introducing NADH to the solution of 1,10 phenanthroline-5,6-dione, the height of the redox peaks decreased (Fig. 2(A), dotted line). Although the intermediate is soluble in water-ethanol mixtures, there is no catalytic current for 1,10-heteroquinone. (see second full paragraph, column 2, page 1270 of Geng et al.)

Accordingly, Geng et al. teaches away from the use of phenanthroline quinone where the atom in the 1 and 10 positions is nitrogen as a mediator for the reaction between a nicotinamide cofactor-dependent enzyme and a nicotinamide cofactor. The mediator(s) recited in claims 18-29 is (are) similar to the mediator(s) recited in claims 1-5, 7-11, 16, and 17, the exception being the positions of the substituent X and the substituent Y. However, the nitrogen atoms in the heteroaromatic rings R<sub>1</sub> and R<sub>2</sub> are in substantially equivalent positions as they are in the compounds of claims 1-5, 7-11, 16, and 17.

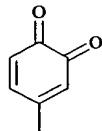
MacFarlane et al. does not disclose or suggest the use of the compounds recited in claims 1 and 18, and the claims depending therefrom, as a mediator for the reaction between nicotinamide cofactor-dependent enzyme and a nicotinamide cofactor.

Carter et al. merely discloses the use of a mediator in general. Carter et al. does not disclose or suggest the use of the compounds recited in claims 1 and 18, and the claims depending therefrom, as a mediator for the reaction between nicotinamide cofactor-dependent enzyme and a nicotinamide cofactor. For the foregoing reasons, it is submitted that the combination of Geng et al., MacFarlane et al., and Carter et al. does not render the claims of this application obvious to one of ordinary skill in the art.

Claims 1-12 and 14 stand rejected under 35 U. S. C. § 103(a) as being unpatentable over Batchelor et al., "AMPEROMETRIC ASSAY FOR THE KETONE BODY 3-HYDROXYBUTYRATE", *Analytica Chimica Acta*, 221 (1989) 289-294, in view of Geng et al., MacFarlane et al., and Carter et al. This rejection is respectfully traversed for the following reasons.

Batchelor et al., "AMPEROMETRIC ASSAY FOR THE KETONE BODY 3-HYDROXYBUTYRATE", *Analytica Chimica Acta*, 221 (1989) 289-294 (hereinafter "Batchelor et al."), discloses a dry-strip electrochemical sensor for the direct measurement of 3-hydroxybutyrate in blood. The sensor utilizes the electrocatalytic oxidation of enzymically generated NADH by the redox mediator 4-methyl-o-quinone. The enzyme 3-hydroxybutyrate dehydrogenase, cofactor NAD<sup>+</sup> and 4-methyl-o-quinone were incorporated into single-use disposable strip electrodes.

Batchelor refers to the use of 4-methyl-o-quinone (4-methyl-1,2-benzoquinone) as a mediator for NADH in the construction of a biosensor electrode for D-3-hydroxybutyrate, which is a ketone. The structure of 4-methyl-o-quinone is:



This mediator is no more suggestive of the mediators recited in the claims of this application than are the mediators disclosed in Geng et al. and Carter et al. Accordingly, for the reasons put forth earlier with respect to the Geng et al. and the Carter et al. references, it is submitted that the combination of Batchelor et al., Geng et al., MacFarlane et al., and Carter et al. does not render the claims of this application obvious to one of ordinary skill in the art.

Claims 4-11 and 14 stand rejected under 35 U. S. C. § 103(a) as being unpatentable over Geng et al., MacFarlane et al., and Carter et al. and further in view of Batchelor et al. This rejection is respectfully traversed for the following reasons.

The reasons for the traversal of this ground of rejection are the same as those discussed previously on pages 11 and 12 of this AMENDMENT AND RESPONSE

with respect to the previous ground of rejection, in which the references Batchelor et al., Geng et al., MacFarlane et al., and Carter et al. were cited.

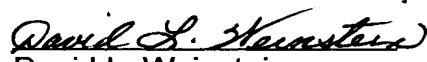
Claims 13 and 15 were rejected under 35 U. S. C. § 103(a) as being unpatentable over Geng et al., MacFarlane et al., and Carter et al. and further in view of Itoh et al., Chemistry Letters, The Chemical Society of Japan, pp. 1583-1586 (1992). This rejection is respectfully traversed for the following reasons. These claims have been cancelled. Accordingly, this rejection can be withdrawn.

Claims 13 and 15 were rejected under 35 U. S. C. § 103(a) as being unpatentable over Batchelor, Geng et al., MacFarlane et al., and Carter et al. and further in view of Itoh et al., Chemistry Letters, The Chemical Society of Japan, pp. 1583-1586 (1992). This rejection is respectfully traversed for the following reasons. These claims have been cancelled. Accordingly, this rejection can be withdrawn.

In view of the foregoing, it is submitted that claims 1-5 and 7-11, as amended, and new claims 16-29 are in condition for allowance, and official Notice of Allowance is respectfully requested.

Abbott Laboratories  
D-377 AP6D-2  
100 Abbott Park Road  
Abbott Park, Illinois 60064-3500  
Telephone: (847) 937-6182

Respectfully submitted,  
Nigel J. Forrow, et al.

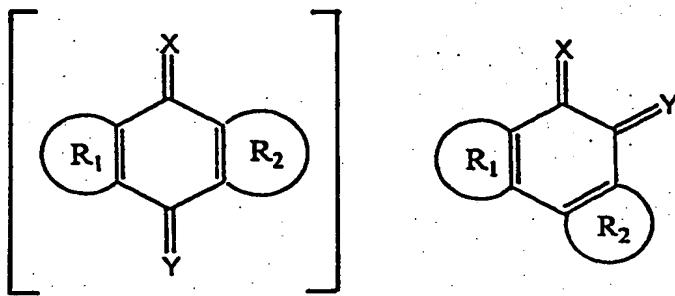
  
David L. Weinstein  
Registration No. 28, 128  
Attorney for Applicants

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

Kindly rewrite claims 1, 3, 4, 7, 9, and 10 as follows:

1. (Once amended) A single use disposable electrode strip for attachment to the signal readout circuitry of a sensor system to detect a current representative of an analyte in an aqueous sample, the strip comprising:

- a) an elongated support having a substantially flat planar surface, adapted for releasable attachment to said readout circuitry;
- b) a first conductor extending along said surface and comprising a conductive element for connection to said readout circuitry; an active electrode on said surface in contact with said first conductor, said active electrode comprising a nicotinamide co-factor-dependent enzyme, a nicotinamide cofactor, and a mediator compound having [one of] the following [two formulae] formula:



where X and Y can independently be oxygen, sulphur, CR<sup>3</sup>R<sup>4</sup>, NR<sup>3</sup>, or NR<sup>3</sup>R<sup>4</sup> or the functional group CZ<sup>1</sup>Z<sup>2</sup>, where Z<sup>1</sup> and Z<sup>2</sup> are electron withdrawing groups; R<sub>1</sub> [and R<sub>2</sub> can independently be a substituted or unsubstituted aromatic or heteroaromatic group] is a six-membered unsubstituted or substituted heteroaromatic ring having nitrogen in the 1-position and R<sub>2</sub> is a six-membered unsubstituted or substituted heteroaromatic ring having nitrogen in the 10-position; and R<sup>3</sup> and R<sup>4</sup> can independently be a hydrogen atom, a hydroxyl group or a substituted or unsubstituted alkyl, aryl, heteroaryl, amino, alkoxy, or aryloxy group, wherein said active electrode is formulated with filler and binder ingredients [such that said electrode gives a monotonic response to concentrations of said analyte between 1 and 8 mM when measurement is made in a kinetic mode in which

simultaneous oxidation and reduction of the mediator occurs during the measurement];

- c) a second conductor extending along said surface, comprising a conductive element for connection to said readout circuitry;
- d) a reference/counter electrode in contact with said second conductor;
- e) said conductors being spaced apart so as not to be in electrical contact and being configured so as not to be brought into electrical contact when said aqueous sample is placed on said strip;
- f) said active electrode and said reference/counter electrode being configured so that both may be simultaneously covered by a small drop of said aqueous sample to provide an electrical conduction path between said electrodes.

3. (Once amended) The electrode strip of claim [2] 1 wherein the co-factor dependent enzyme is glucose dehydrogenase.

4. (Once amended) The electrode strip of claim [2] 1 wherein the co-factor dependent enzyme is hydroxybutyrate dehydrogenase.

7. (Twice amended) The process of claim [6] 5 wherein the current observed during the measurement period is linearly related to the concentration of the analyte in the sample.

9. (Once amended) The process of claim [8] 5 wherein the co-factor dependent enzyme is glucose dehydrogenase.

10. (Once amended) The process of claim [8] 5 wherein the co-factor dependent enzyme is hydroxybutyrate dehydrogenase.